

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

RECEIVED
CENTRAL FAX CENTER

APR 03 2007

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-15 (Cancelled).

16. (Previously Presented) An isolated nucleic acid comprising the nucleotide sequence encoding the effector domain of the binding molecule as claimed in claim 32, wherein said nucleic acid is DNA.

17. (Previously Presented) An isolated nucleic acid comprising the nucleotide sequence encoding the binding molecule as claimed in claim 32, wherein said nucleic acid is DNA.

18. (Previously Presented) The nucleic acid as claimed in claim 16 which is a replicable vector.

19. (Previously Presented) The nucleic acid as claimed in claim 18 wherein the nucleotide sequence is operably linked to a promoter.

20. (Currently Amended) A An isolated host cell comprising or transformed with the vector of claim 19.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

21. (Currently Amended) A process for producing a binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain having an amino acid sequence homologous to a constant domain of a human immunoglobulin heavy chain;

wherein the binding molecule is capable of binding the target molecule without triggering significant complement dependent lysis, or cell mediated destruction of the target, and the effector domain is capable of specifically binding Fc γ RIIb and optionally FcRn,

and wherein the effector domain comprises a chimeric C_H2 domain which is derived from two or more human immunoglobulin heavy chain C_H2 domains, which human immunoglobulins are selected from IgG1, IgG2 and IgG4,

and wherein the effector domain has a reduced affinity for Fc γ RI, Fc γ RIIa and Fc γ RIII and a reduced ability to mediate complement lysis by comparison with said constant domain of a human immunoglobulin heavy chain;

the process comprising the steps of modifying a nucleotide sequence encoding a first human immunoglobulin heavy chain C_H2 domain such that 2, or 3 or 4 amino acids in at least 1 region of the C_H2 domain correspond to the amino acids from a second human immunoglobulin heavy chain C_H2 domain,

wherein said modification introduces the following blocks of amino acids at the stated positions: 233P, 234V, 235A, 236G, 327G, 330S and 331S numbered with respect to the EU numbering system of Kabat

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

and wherein said chimeric C_H2 domain is at least 98% identical to a C_H2 sequence (residues 231-340) from human IgG1, IgG2 or IgG4 having said modified amino acids introducing into a host cell a vector comprising said modified nucleotide sequence, culturing said host cell under conditions such that said binding molecule is produced, and isolating said binding molecule from said cell culture.

22. (Cancelled).

23. (Currently Amended) A method of binding ~~the a~~ target molecule, said method ~~that the binding molecule of claim 32 is capable of binding, which method comprises~~ comprising contacting said target molecule with said binding molecule of claim 32 under conditions to allow binding.

24. (Currently Amended) The method of claim 23 wherein the effector domain specifically binds FcγRIIb, which binding causes inhibition of one or more of: B cell activation; mast cell degranulation; and phagocytosis.

25. (Currently Amended) The method of claim 23 to ~~prevent, inhibit, or otherwise~~ ~~interfere with~~ the binding of a second binding molecule to the target molecule.

26. (Previously Presented) The method of claim 25 wherein the second binding molecule is an antibody.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

27. (Previously Presented) The method of claim 23 wherein the target molecule is selected from the group consisting of the RhD antigen of red blood cells; a human platelet antigen (HPA); a neutrophil antigen; a T-cell receptor; an integrin; a glomerular basement membrane (GBM) collagen type IV; a Der P1; VAP-1; laminin; lutheran; platelet glycoprotein VI; and platelet glycoprotein Ia/IIa.

28. (Currently Amended) The method of claim 23 wherein said target molecule ~~contacting~~ is effected in a patient suffering from a disorder selected from the group consisting of:

- i) Graft-vs-host disease, host-vs-graft disease, organ transplant rejection, bone-marrow transplant rejection, autoimmune vasculitis, arthritis or asthma, wherein the target molecule is a T-cell receptor;
- ii) autoimmune haemolytic anaemia or autoimmune thrombocytopenia, wherein the target molecule is selected from the group consisting of red blood cell Rhesus antigens D,C,c,E and e, the Kell (K1) antigen and platelet glycoprotein GPIIb/IIIa and GPIb/IX/V;
- iii) foetal/neonatal alloimmune thrombocytopenia, wherein the target molecule is human platelet antigen (HPA)-1a or platelet glycoprotein IIIa;
- iv) dust mite allergy, wherein the target molecule is Der P1 protein of the house dust mite *Dermatophagoides pteronyssinus*;
- v) Crohn's, wherein the target molecule is VAP-1;
- vi) haemolytic disease of the newborn (HDN), wherein the target molecule is selected from the group consisting of red blood cell Rhesus antigens D,C,c,E and e, and the Kell (K1) antigen;

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

- vii) Goodpastures, wherein the target molecule is non-collagenous (NC1) domain of $\alpha 3(\text{IV})$ collagen;
- viii) sickle cell anaemia, wherein the target molecule is selected from the group consisting of: thrombospondin, laminin and lutheran; and
- ix) coronary artery occlusion, wherein the target molecule is selected from the group consisting of integrin $\alpha_2\beta_1$ (platelet glycoprotein Ia/IIa) and non-integrin platelet glycoprotein VI.

29. (Currently Amended) The method of claim 23 wherein the contacting step is a step of administering the binding molecule is administered to a patient, or optionally to the mother of the patient in cases where the patient is an unborn infant, ~~to the mother of the patient.~~

Claim 30 (Cancelled).

31. (Withdrawn) An oligonucleotide selected from:

MO22BACK: 5' TCT CCA ACA AAG GCC TCC CGT CCT CCA TCG AGA AAA 3' (SEQ ID NO:16)

MO22: 5' TTT TCT CGA TGG AGG ACG GGA GGC CTT TGT TGG AGA 3' (SEQ ID NO:17)

MO7BACK: 5' TCC TCA GCA CCT CCA GTC GCG GGG GGA CCG TCA GTC 3' (SEQ ID NO:18)

MO21: 5' GAC TGA CGG TCC CGC GAC TGG AGG TGC TGA GGA 3' (SEQ ID NO:19)

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

32. (Currently Amended) A binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain having an amino acid sequence homologous to a constant domain of a human immunoglobulin heavy chain;

wherein the binding molecule is capable of binding the target molecule without triggering significant complement dependent lysis, or cell mediated destruction of the target, and the effector domain is capable of specifically binding Fc γ RIIb and optionally FcRn,

and wherein the effector domain comprises a chimeric C_H2 domain which is derived from two or more human immunoglobulin heavy chain C_H2 domains, which human immunoglobulins are selected from IgG1, IgG2 and IgG4,

and wherein the effector domain has a reduced affinity for Fc γ RI, Fc γ RIIa and Fc γ RIII and a reduced ability to mediate complement lysis by comparison with said constant domain of a human immunoglobulin heavy chain

and wherein the chimeric C_H2 domain is a human immunoglobulin heavy chain C_H2 domain which has the following blocks of amino acids at the stated positions: 233P, 234V, 235A, 236G, 327G, 330S and 331S numbered with respect to the EU numbering system of Kabat, and is at least 98% identical to a C_H2 sequence (residues 231-340) from human IgG1, IgG2 or IgG4 having said modified amino acids.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

33. (Previously Presented) The binding molecule as claimed in claim 32 wherein the chimeric C_H2 domain consists of G1Δac (SEQ ID NO:3) or G4Δc (SEQ ID NO:12) as shown in Figure 17.

Claims 34-36 (Cancelled).

37. (Previously Presented) The binding molecule as claimed in claim 32 wherein the binding domain derives from a different source to the effector domain.

38. (Currently Amended) The binding molecule as claimed in claim 32 wherein the target molecule is selected from the group consisting of the RhD antigen of red blood cells; a human platelet antigen (HPA); a neutrophil antigen; a T-cell receptor; an integrin; a glomerular basement membrane (GBM) collagen type IV; a Der P1; VAP-1; laminin; lutheran; platelet glycoprotein VI; and platelet glycoprotein Ia/IIa.

39. (Previously Presented) The binding molecule as claimed in claim 38 wherein the binding domain is the binding site of an antibody selected from the group consisting of anti-CD52; anti-RhD; anti-HPA-1a; anti-VAP-1; murine anti-α3 (IV) NC1; anti-CD3; anti-Der p I; anti-laminin; and anti-lutheran.

40. (Previously Presented) A preparation comprising the binding molecule as claimed in claim 32 plus a pharmaceutically acceptable carrier.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

41. (Previously Presented) A binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain having an amino acid sequence homologous to a constant domain of a human immunoglobulin heavy chain;

wherein the binding molecule is capable of binding the target molecule without triggering significant complement dependent lysis, or cell mediated destruction of the target, and the effector domain is capable of specifically binding Fc γ RIIb and optionally FcRn,

and wherein the effector domain comprises a chimeric C_H2 domain which is derived from two or more human immunoglobulin heavy chain C_H2 domains, which human immunoglobulins are selected from IgG1, IgG2 and IgG4,

and wherein the effector domain has a reduced affinity for Fc γ RI, Fc γ RIIa and Fc γ RIII and a reduced ability to mediate complement lysis by comparison with said constant domain of a human immunoglobulin heavy chain

and wherein the chimeric C_H2 domain is a human immunoglobulin heavy chain C_H2 domain which has the following blocks of amino acids at the stated positions: 233P, 234V, 235A and no residue at 236, 327G, 330S and 331S, numbered with respect to the EU system of Kabat, and is at least 98% identical to a C_H2 sequence (residues 231-340) from human IgG1 or IgG2 having said modified amino acids.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

42. (Previously Presented) The binding molecule as claimed in claim 41 wherein the chimeric C_H2 domain consists of G1Δab (SEQ ID NO:1) or G2Δa (SEQ ID NO:2) as shown in Figure 17.

Claims 43-45 (Cancelled).

46. (Previously Presented) The binding molecule as claimed in claim 41 wherein the binding domain derives from a different source to the effector domain.

47. (Currently Amended) The binding molecule as claimed in claim 41 wherein the target molecule is selected from the group consisting of the RhD antigen of red blood cells; a human platelet antigen (HPA); a neutrophil antigen; a T-cell receptor; an integrin; a glomerular basement membrane (GBM) collagen type IV; a Der P1; VAP-1; laminin; lutheran; platelet glycoprotein VI; and platelet glycoprotein Ia/IIa.

48. (Previously Presented) The binding molecule as claimed in claim 47 wherein the binding domain is the binding site of an antibody selected from the group consisting of anti-CD52; anti-RhD; anti-HPA-1a; anti-VAP-1; murine anti-α3 (IV) NC1; anti-CD3; anti-Der p I; anti-laminin; and anti-lutheran.

49. (Previously Presented) A preparation comprising the binding molecule as claimed in claim 41 plus a pharmaceutically acceptable carrier.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

50. (Previously Presented) An isolated nucleic acid comprising the nucleotide sequence encoding the effector domain of the binding molecule as claimed in claim 41, wherein said nucleic acid is DNA.

51. (Previously Presented) An isolated nucleic acid comprising the nucleotide sequence encoding the binding molecule as claimed in claim 41, wherein said nucleic acid is DNA.

52. (Previously Presented) The nucleic acid as claimed in claim 50 which is a replicable vector.

53. (Previously Presented) The nucleic acid as claimed in claim 52 wherein the nucleotide sequence is operably linked to a promoter.

54. (Currently Amended) A An isolated host cell comprising or transformed with the vector of claim 53.

55. (Previously Presented) A process for producing a binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain having an amino acid sequence homologous to a constant domain of a human immunoglobulin heavy chain;

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

wherein the binding molecule is capable of binding the target molecule without triggering significant complement dependent lysis, or cell mediated destruction of the target, and the effector domain is capable of specifically binding $\text{Fc}\gamma\text{RIIb}$ and optionally FcRn ,

and wherein the effector domain comprises a chimeric $\text{C}_{\text{H}2}$ domain which is derived from two or more human immunoglobulin heavy chain $\text{C}_{\text{H}2}$ domains, which human immunoglobulins are selected from IgG1, IgG2 and IgG4,

and wherein the effector domain has a reduced affinity for $\text{Fc}\gamma\text{RI}$, $\text{Fc}\gamma\text{RIIa}$ and $\text{Fc}\gamma\text{RIII}$ and a reduced ability to mediate complement lysis by comparison with said constant domain of a human immunoglobulin heavy chain;

the process comprising the steps of modifying a nucleotide sequence encoding a first human immunoglobulin heavy chain $\text{C}_{\text{H}2}$ domain such that 2, 3 or 4 amino acids in at least 1 region of the $\text{C}_{\text{H}2}$ domain correspond to the amino acids from a second human immunoglobulin heavy chain $\text{C}_{\text{H}2}$ domain,

wherein said modification introduces the following blocks of amino acids at the stated positions: 233P, 234V, 235A and no residue at 236 and 327G, 330S and 331S numbered with respect to the EU numbering system of Kabat

and wherein said chimeric $\text{C}_{\text{H}2}$ domain is at least 98% identical to a $\text{C}_{\text{H}2}$ sequence (residues 231-340) from human IgG1 or IgG2 having said modified amino acids

introducing into a host cell a vector comprising said modified nucleotide sequence,
culturing said host cell under conditions such that said binding molecule is produced, and
isolating said binding molecule from said cell culture.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

56. (Previously Presented) The process as claimed in claim 55 wherein 2 amino acids in 1 region of the C_H2 domain are modified to the corresponding amino acids from the second human immunoglobulin heavy chain C_H2 domain.

57. (Currently Amended) A method of binding the target molecule, ~~that the binding molecule of claim 41 is capable of binding, which~~ said method comprises contacting said target molecule with said binding molecule of claim 41 under conditions to allow binding.

58. (Currently Amended) The method of claim 57 wherein the effector domain specifically binds FcγRIIb, which binding causes inhibition of one or more of: B cell activation; mast cell degranulation; and phagocytosis.

59. (Currently Amended) The method of claim 57 to ~~prevent, inhibit, or otherwise interfere with~~ the binding of a second binding molecule to the target molecule.

60. (Previously Presented) The method of claim 59 wherein the second binding molecule is an antibody.

61. (Currently Amended) The method of claim 57 wherein the target molecule is selected from: the group consisting of the RhD antigen of red blood cells; an HPA alloantigen of platelets; a neutrophil antigen; a T-cell receptor; integrin; GBM collagen; Der P1; HPA-1a; VAP-1; laminin; lutheran; platelet glycoprotein VI; and platelet glycoprotein Ia/IIa.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

62. (Currently Amended) The method of claim 57 wherein said target molecule ~~contacting~~ is effected in a patient suffering from a disorder selected from the group consisting of:

- i) Graft-vs-host disease, host-vs-graft disease, organ transplant rejection, bone-marrow transplant rejection, autoimmune vasculitis, arthritis or asthma, wherein the target molecule is a T-cell receptor;
- ii) autoimmune haemolytic anaemia or autoimmune thrombocytopenia, wherein the target molecule is selected from the group consisting of red blood cell Rhesus antigens D,C,c,E and e, the Kell (K1) antigen and platelet glycoprotein GPIIb/IIIa and GPIb/IX/V;
- iii) foetal/neonatal alloimmune thrombocytopenia, wherein the target molecule is human platelet antigen (HPA)-1a or platelet glycoprotein IIIa;
- iv) dust mite allergy, wherein the target molecule is Der P1 protein of the house dust mite *Dermatophagoides pteronyssinus*;
- v) Crohn's, wherein the target molecule is VAP-1;
- vi) haemolytic disease of the newborn (HDN), wherein the target molecule is selected from the group consisting of red blood cell Rhesus antigens D,C,c,E and e, and the Kell (K1) antigen;
- vii) Goodpastures, wherein the target molecule is non-collagenous (NC1) domain of $\alpha 3(\text{IV})$ collagen;
- viii) sickle cell anaemia, wherein the target molecule is selected from the group consisting of: thrombospondin, laminin and lutheran; and
- ix) coronary artery occlusion, wherein the target molecule is selected from the group consisting of integrin $\alpha_2\beta_1$ (platelet glycoprotein Ia/IIa) and non-integrin platelet glycoprotein VI.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

63. (Currently Amended) The method of claim 57 wherein the contacting step is a step of administering the binding molecule ~~is administered~~ to a patient, or optionally to the mother of the patient ~~in cases where the patient is an unborn infant, to the mother of the patient.~~

64. (Previously Presented) The binding molecule as claimed in claim 39 wherein the anti-CD52 binding domain is CAMPATH-1; the anti-RhD is FOG1; the anti-Der p I is 2C7; the anti-CD3 is YTH12.5.

65. (Previously Presented) The binding molecule as claimed in claim 48 wherein the anti-CD52 binding domain is CAMPATH-1; the anti-RhD is FOG1; the anti-Der p I is 2C7; the anti-CD3 is YTH12.5.

66.-67. (Cancelled).

68. (Previously Presented) The method as claimed in claim 27 wherein the HPA is HPA-1a.

69. (Previously Presented) The binding molecule as claimed in claim 38 wherein the HPA is HPA-1a.

70. (Previously Presented) The binding molecule as claimed in claim 47 wherein the HPA is HPA-1a.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

71. (New) A binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain comprising chimeric C_H2 domain which consists of G1Δac (SEQ ID NO:3) as shown in Figure 17.

72. (New) A binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain comprising chimeric C_H2 domain which consists of G4Δc (SEQ ID NO:12) as shown in Figure 17.

73. (New) A binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain comprising chimeric C_H2 domain which consists of G1Δab (SEQ ID NO:1) as shown in Figure 17.

74. (New) A binding molecule which is a recombinant polypeptide comprising:

(i) a binding domain capable of binding a target molecule, which binding domain is the binding site of an antibody, and

(ii) an effector domain comprising chimeric C_H2 domain which consists of G2Δa (SEQ ID NO:2) as shown in Figure 17.

ARMOUR et al.
Appl. No. 09/674,857
April 3, 2007

75. (New) An isolated nucleic acid comprising the nucleotide sequence encoding the effector domain of the binding molecule as claimed in claim 71, wherein said nucleic acid is DNA.

76. (New) An isolated nucleic acid comprising the nucleotide sequence encoding the effector domain of the binding molecule as claimed in claim 72, wherein said nucleic acid is DNA.

77. (New) An isolated nucleic acid comprising the nucleotide sequence encoding the effector domain of the binding molecule as claimed in claim 73, wherein said nucleic acid is DNA.

78. (New) An isolated nucleic acid comprising the nucleotide sequence encoding the effector domain of the binding molecule as claimed in claim 74, wherein said nucleic acid is DNA.